

(12) UK Patent Application (19) GB (11) 2 147 698 A

(43) Application published 15 May 1985

(21) Application No 8426736

(22) Date of filing 16 Nov 1982

Date lodged 23 Oct 1984

(30) Priority data

(31) 8134578 (32) 17 Nov 1981 (33) GB

(60) Derived from Application No 8232685 under Section 15(4) of the Patents Act 1977

(71) Applicant
Unilever PLC (United Kingdom),
Unilever House, Blackfriars, London EC4P 4BQ

(72) Inventors
Lionel Frederick Shapland Albon,
Anthony John Winterson

(74) Agent and/or Address for Service
T. C. Stancliffe,
Patent Division, Unilever PLC, PO Box 68, London EC4

(51) INT CL⁴
G01N 33/543 B01L 3/00

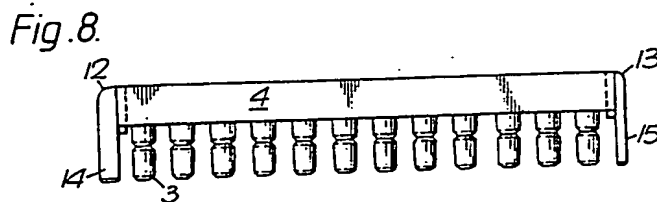
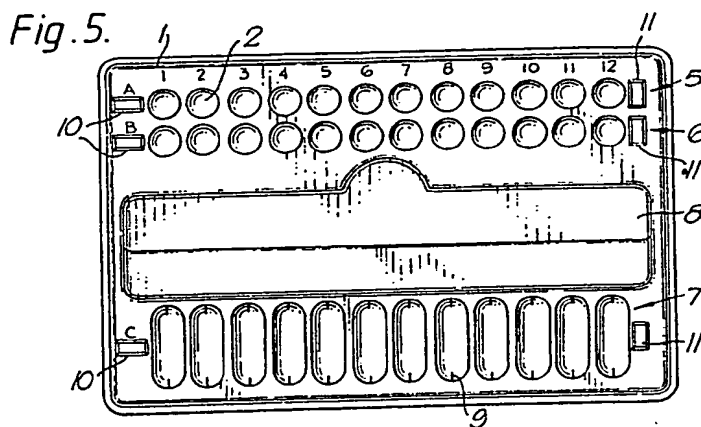
(52) Domestic classification
G1B CB CC
U1S 1059 1068 G1B

(56) Documents cited
None

(58) Field of search
G1B

(54) Test apparatus for immunoassay

(57) In apparatus for clinical chemical test procedures such as immunoassay and comprising in combination a holder 4 (Fig. 8) bearing a number of inserts for reaction wells, and a tray 1 (Fig. 5) having a plurality of reaction wells arranged in a pattern complementary to the pattern of a plurality of the inserts, so that several of the inserts can be introduced at once into the corresponding reaction wells, the holder and the tray have complementary locating means such as 10, 11, 14, 15 to allow insertion of the inserts in a desired orientation into the wells, and to prevent incorrect insertion in inverse orientation. Preferably the inserts bear a specific sensitisation, e.g. an immunological sensitisation, reagents for the assay reaction are dosed and stabilised in the wells, and a sealing cover (not shown) retains the reagents in stable form in storage before use.



GB 2 147 698 A

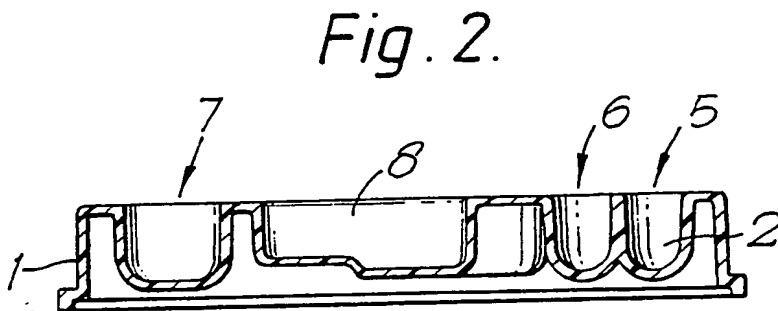
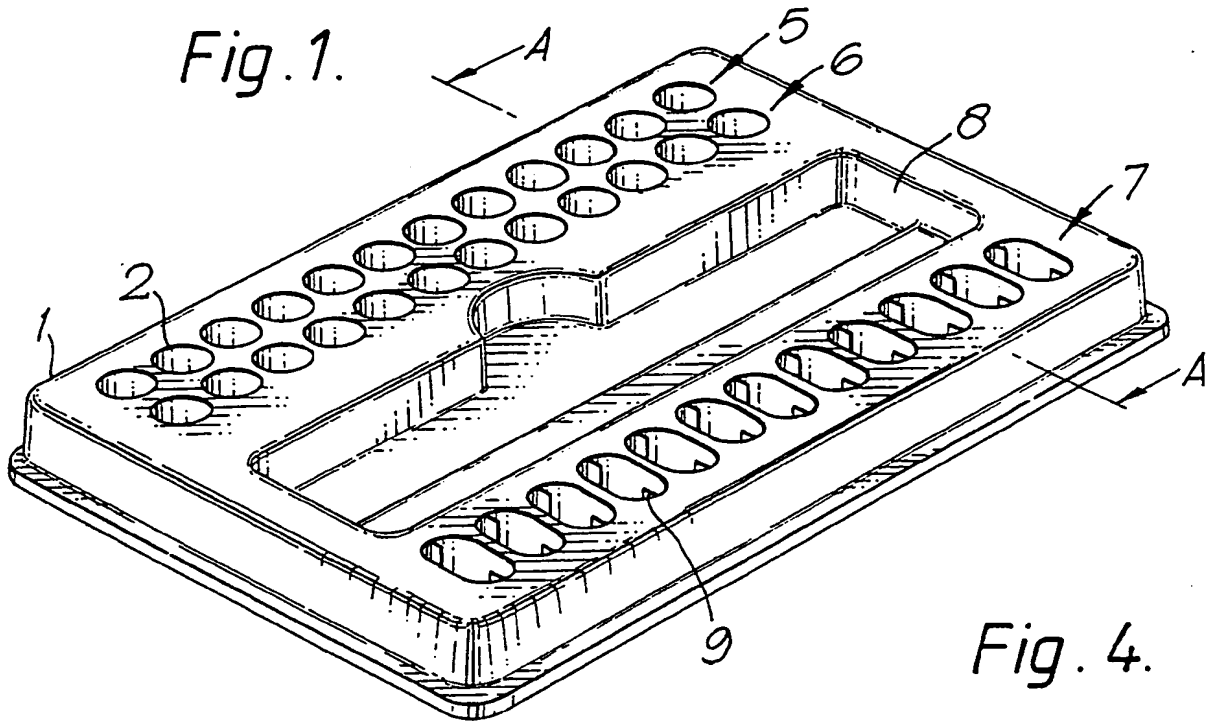


Fig. 4.

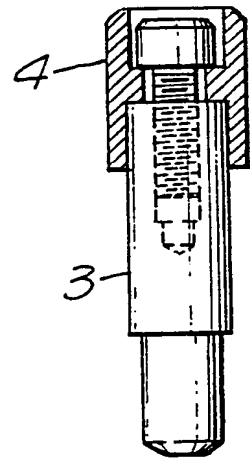


Fig. 3.

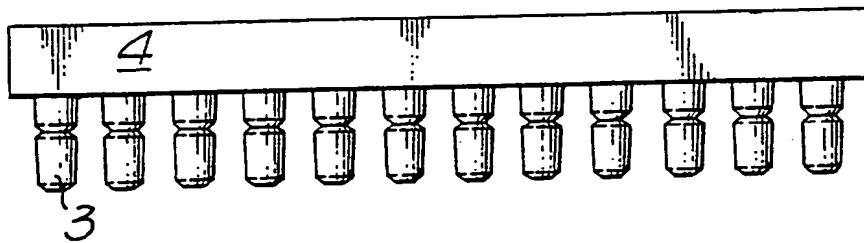


Fig. 5.

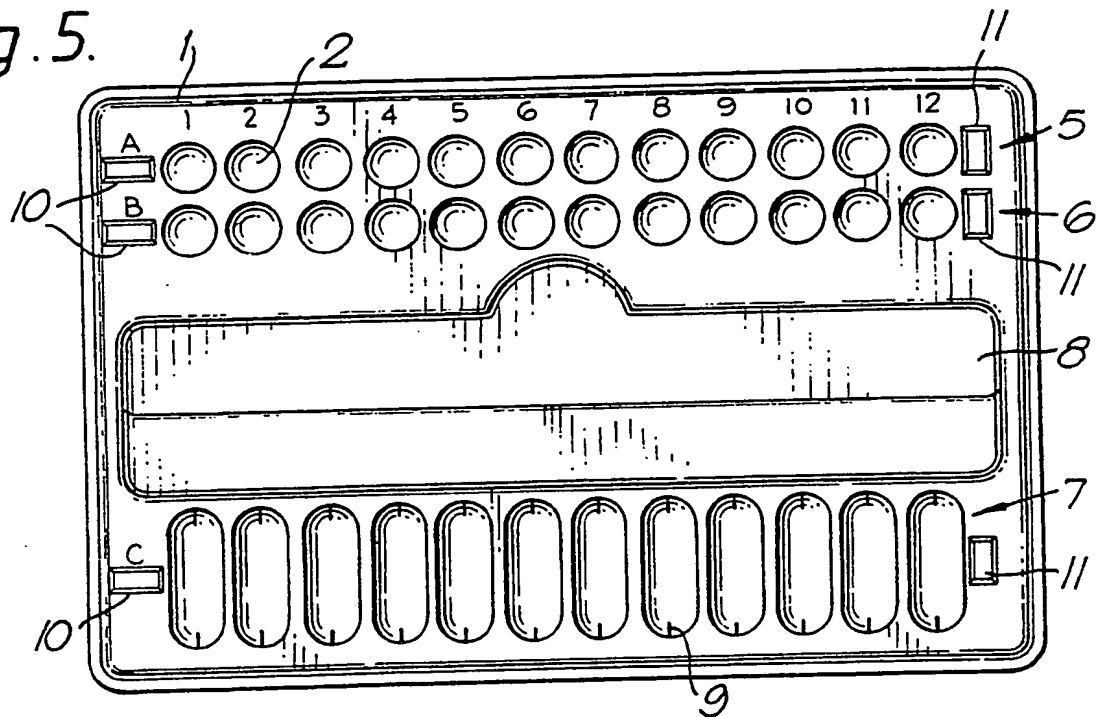


Fig. 6.

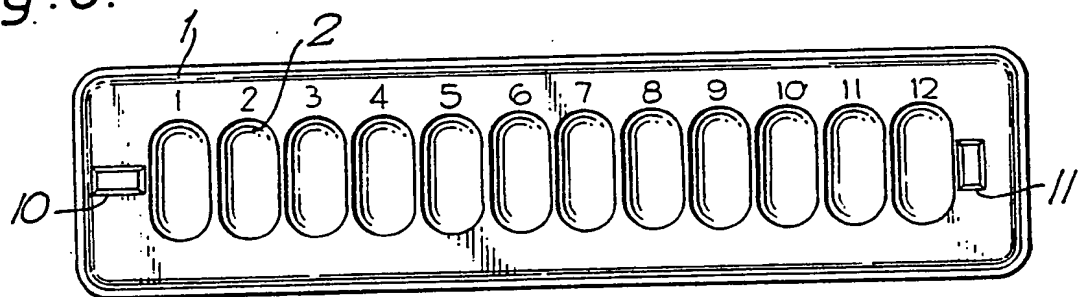


Fig. 7.

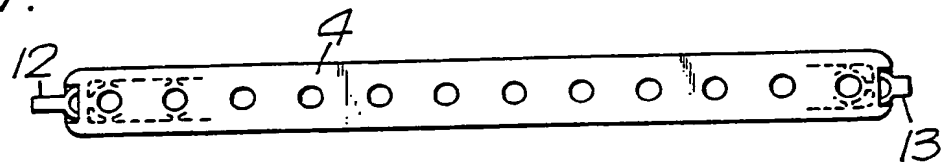
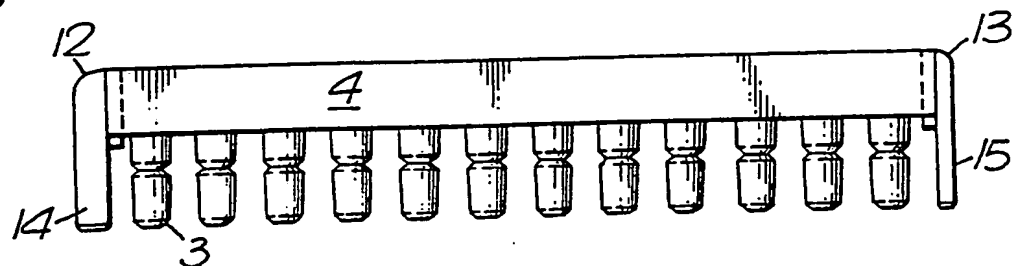


Fig. 8.



SPECIFICATION

Micro-scale test apparatus suitable for clinical chemistry

5 This invention relates to micro-scale test apparatus suitable for clinical chemical test procedures.

It is already well known to use microtitre trays in clinical diagnostic procedures for a multiplicity of
10 applications, e.g. for the microbial culture of septic samples from patients as in microbial identification and sensitivity testing, and for assay procedures related to body fluid samples, e.g. immunoassay.

One form of such a microtitre tray consists of a
15 plastics (e.g. injection-moulded) tray containing eight rows of twelve formed microwells each with a liquid capacity of a fraction of a millilitre.

Various attempts have been made to improve the convenience in use of such micro-scale apparatus.

20 Examples are described by e.g. H. Park, J. Immunol. Methods, 20 (1978) 349-355, (through-passage receptacles); GB Specification 2,012,955 (trapped beads within receptacles); and P. Felgner (Zb1. Bakt. Hyg. I Abt. Orig. A. 240, 112-117 (1978), (assemblies
25 of reaction tubes and clamped inserts).

It is also disclosed in GB Patent Specification No. 1,414,479, that radioimmunoassay reactions can be carried out using (a) a sample receptacle and (b) a reagent-carrying member with a handle inserted into
30 the receptacles during the course of the radioimmunoassay procedure.

According to the present invention we provide a convenient apparatus applicable to numerous forms of clinical test procedure and especially immunoassay, for example enzyme-linked immunoassay and
35 fluorescence-linked immunoassay. The apparatus shows a combination of a holder bearing a number of inserts for reaction wells, and a tray which has a main recess, for the holder and the inserts borne
40 thereon, when not in use or when stored ready for use, and a plurality of reaction wells arranged in a pattern complementary to the pattern of a plurality of the inserts, so that several of the inserts can be introduced at once into the corresponding reaction
45 wells. Conveniently, the tray can comprise more than one series of reaction wells, so that a plurality of the inserts can be introduced first into one series of wells on the tray, and then into one or more successive series, accordingly as the course of the
50 particular reaction and the convenience of the user requires.

Also provided by the invention is an apparatus applicable to the same test procedures, showing a combination of a holder bearing a plural number of
55 inserts for reaction wells, and a tray with a plurality of reaction wells arranged in a pattern complementary to the pattern of a plurality of the inserts, so that several of the inserts can be introduced at once into the corresponding reaction wells, the holder and the
60 tray bearing complementary locating means to allow insertion of the inserts in a desired orientation of matching between the inserts and the wells, and to prevent incorrect insertion in the inverse orientation.

The complementary locating means can for example
65 take the form of a plug or socket, e.g. of slot shape, at

one or both ends of a row of wells, and a complementary socket or plug at the complementary position fixed to the block of inserts. This is of use in preventing incorrect matching of the parts of the
70 apparatus by the user during the performance of the tests and assays for which the apparatus is designed.

It will often be convenient to provide the inserts with a shape which substantially complements the
75 shape of the reaction wells, so that reaction liquid contained in a well of for example round cross-section in plan and a flat or rounded bottom, is constrained to a shell-like distribution when the complementary insert is also introduced into the
80 well.

Preferred embodiments of the invention are illustrated by way of example in the accompanying diagrammatic drawings, and further details of them are described below.

85 In the drawings:

Figure 1 shows a perspective view of a tray which forms part of one embodiment of apparatus according to the present invention.

Figure 2 shows a sectional view on line A-A in
90 *Figure 1*.

Figure 3 shows in front view a complementary holder bearing inserts for reaction wells, which together with the tray of *Figures 1* and *2* forms one embodiment of apparatus according to the present
95 invention.

Figure 4 shows a sectional view of a holder of the kind shown in *Figure 3*, illustrating one mode of attachment between holder and inserts.

Figure 5 shows in plan view another tray which
100 forms part of a second embodiment of apparatus according to the present invention.

Figure 6 shows in plan view a third tray which forms part of a third embodiment of apparatus according to the present invention.

105 *Figures 7* and *8* respectively show in plan and front view, and in fragmentary diagrammatic form, a holder bearing inserts for reaction wells, which is adapted to form, in combination with either the tray of *Figure 5* or the tray of *Figure 6*, a respective
110 embodiment of apparatus according to the present invention.

One suitable example of the apparatus is illustrated by *Figures 1-4* of the drawings.

Referring to *Figures 1-2*, a plastics tray 1 contains a
115 plurality of plastics wells (e.g. as shown at 2) of about 7 mm internal diameter and about 9 mm spacing centre-to-centre. The wells can be about 9 mm deep with substantially flat or substantially hemispherical bottoms. Substantially complementary-shaped inserts 3, shown in *Figures 3-4*, of about 5mm internal diameter and about 8 mm depth are fixedly mounted in a row on a suitable holder 4.
120 Their size and spacing permits all the inserts in the row to be inserted together into one of the rows of wells in tray 1. The close proximity of the walls of the wells and the insert means that the liquid in the well is constrained between them to a shell-like distribution, and its thinness tends to accelerate the progress of the reactions taking place between the insert
125 surface sensitisation and the liquid.
130

The number of inserts arranged on holder 4 can be as desired, and conveniently can match the number of wells in a row in Tray 1. Figure 3 shows a holder with twelve attached inserts.

- 5 If desired, the inserts can be removably mountable on holder 4. The mounting can be arranged in any desired way, e.g. by a stud-and-socket press-fitting, or a screw mounting, or by adhesive bonding. Alternatively the inserts and holder can be integrally
10 formed, e.g. by moulding in any suitable material, such as for example nylon or polystyrene. Figure 4 schematically shows one suitable screw-fitting attachment between insert 3 and holder 4.

- The ends of the inserts which, on insertion into the
15 wells, reach deepest down towards the bottom, are preferably sensitised with a specific binding agent, e.g. to form an immunosorbent. Methods of carrying out this sensitisation are described for example in our earlier European patent application No. 0 014
20 530 and references cited therein, as well as in the publications cited above in this specification. These ends of the inserts can if desired bear corrugations, ribs, grooves or the like to increase the available reaction surface area.

- 25 As shown in the drawings (Figure 1), tray 1 has three rows of wells, 5, 6 and 7. Each row is arranged to accommodate the holder 4 and inserts. In use the holder 4 and inserts can for example be dipped into row 5 and kept in contact with its liquid contents,
30 then removed and washed if desired, e.g. in running water, before being dipped into row 6 for a further reaction, followed if desired by a further wash before insertion into row 7 for a further reaction.

- As shown in the drawing, the wells of row 7 are
35 laterally elongated to an elliptical or ovoidal shape, so that a greater liquid volume can be held during reaction with the sensitised surface of the insert. If desired a rocking agitation can be imparted to the inserts and well contents during reaction in this
40 shape of well. One optional feature also shown in Figure 1 is the presence of one or a pair of ribs or fins standing outwards from the end walls of the elongate wells of row 7. One such fin is shown as 9 in Figure 1. Such fins can improve the stability of the
45 block of inserts 3 mounted on holder 4 when inserted into the elongate wells of row 7.

- The arrangement shown is of course susceptible to any desired permutation and recombination of its various described features, but is particularly applicable to immunoassay in which case the wells of row
50 5 and/or 6 can contain one or more sample liquids and/or reagent liquids containing labelled binding reagents either together or separately accordingly as the assay format requires, and the wells of row 7 can
55 contain either a substrate for enzyme to be bound to the inserts, where the assay is an enzyme-linked binding assay, or a dissolution medium for bound radioactive or fluorescent label where the assay is a radioassay or a fluorescence assay.

- 60 The tray 1 also contains a large well 8 for storage of the mounted inserts and handle 4. Overall, a foil or other sealing cover sheet can be provided to promote good storage stability for the sensitised inserts and any ancillary test reagents dosed in wells of
65 rows 5, 6 and 7.

- Accordingly, it can be seen that the invention includes a prepared kit for enzyme-linked or fluorescence-linked immunoassay in which apparatus according to the invention described herein has
70 reagents for immunoassay dosed into the wells of the tray, and suitable immunological sensitisation applied to the surface of the inserts carried on the holder. For example, row 5 and/or row 6 of the wells can have dosed therein a stabilised and calibrated
75 small quantity of a substance to be assayed or a specific binding partner therefor, in each case conjugated to a marker substance. Well 8 can contain a block of inserts mounted on a holder for insertion into the wells, the inserts being sensitised by
80 immobilisation thereon of small quantities of either the substance to be assayed or a specific binding partner therefor. Row 7 of the wells can contain substrate for an enzyme when used as the marker substance. The choice of reagents is illustrated in
85 respect of a wide range in for example European Patent Application Nos. 0 014 530, 0 042 755, 0 038 642 and 0 045 103, UK Patent Application No. 2 074 727 and Belgian Patent No. 889 855. Suitable preservation methods are for example those given in UK
90 Patent Specification No. 1 574 707.

- For example, in connexion with an enzyme-linked assay for an antigen such as B₂-microglobulin, to be carried out in a manner which is known so far as concerns the reagents, one row of wells 5 and/or 6
95 can be dosed with calibrated small quantities of the antigen, e.g. the B₂-microglobulin, in conjugated form linked to a marker substance, e.g. alkaline phosphatase. A phosphatase substrate can be dosed into wells of row 7, and the inserts stored in well 8
100 can have antibody to the antigen immobilised upon them in standardised (calibrated) small quantity. For satisfactory storage, the whole assembly is preferably covered with an adhesive sealing cover sheet, (not shown in the drawings).

- 105 A further embodiment of the invention is shown by Figure 5 in plan view: the tray with microtitre wells and receptacle well for a block of inserts carried on a holder generally resembles that of Figures 1 and 2, with the following further features.
110 Each row of wells is provided with means for locating a complementary block of inserts in a desired orientation of matching between the inserts and the wells, so that the block of inserts will not fit if the user tries to insert them in the incorrect (re-
115 versed) orientation. Two unlike sockets 10 and 11 are provided adjacent each row of wells. In the embodiments shown they take the form of slots oriented respectively parallel and perpendicular to the length of the row of wells.

- 120 An embodiment of the invention in which this feature is associated with a tray having a single row of wells is shown in plan view in Figure 6.

- Figures 7 and 8 show in plan and (fragmentary) front view the corresponding block of inserts 3
125 carried on holder 4. In Figure 8 one insert only is shown in full: the dotted axes represent the locations of the remaining eleven inserts as in Figure 3. To correspond with the slots 10 and 11 of the trays shown in Figures 5 and 6, the insert block of Figures
130 7 and 8 has at each end a complementary plug 12

and 13. Plugs 12 and 13 are fixed to holder 4, either by integral formation therewith or permanent adhesive, or else they can be a secure but removable press or push fit. Each plug 12 and 13 has a lower portion 14 and 15 which mates with the corresponding slot in the tray of wells but will not mate with the slots at the opposite ends of the rows of wells. Thus the inserts 3 can only be inserted into the row of wells in one sense. This feature is especially convenient where different assay conditions are being used in different wells of any row, and allow separate identification of the reaction sequence by column. In variants of the arrangements shown in Figures 5-8, within the scope of the invention, the complementary plugs and sockets can have other forms than those shown by numerals 10-15. For example it is particularly advantageous to arrange the sockets as slots having their length oriented perpendicular to the length direction of the row of wells, with the length of each slot longer than the width of the wells, and the slots at each end differently spaced from the first or last well of the row of wells. The plugs are of complementary shape, size and orientation. This prevents any kind of misplacement during use. For this purpose, it is convenient to mark any or all of the columns of wells, the rows of wells and the inserts of holder with letters, numbers, colours or other indicia to aid the user in carrying out tests by the help of the apparatus.

The features described above can of course be used in any permutation or combination, which will be apparent to the instructed reader, for the purpose of adapting the form of the test apparatus to the format of reaction schemes appropriate to any known adsorption or other assay to be undertaken.

CLAIMS

1. Apparatus suitable for carrying out clinical chemical tests and assays such as immunoassays, comprising a tray of microtitre wells and characterised by comprising in combination a holder bearing a plural number of inserts for reaction wells, and a tray with a plurality of reaction wells arranged in a pattern complementary to the pattern of a plurality of the inserts, so that several of the inserts can be introduced at once into the corresponding reaction wells, the holder and the tray bearing complementary locating means to allow insertion of the inserts in a desired orientation between the inserts and the wells, and to prevent insertion in the inverse orientation.

2. Apparatus according to claim 1 or 8, characterised by unlike sockets adjacent each end of a row of wells in the tray, and complementary plugs secured to the holder.

3. Apparatus suitable for carrying out clinical chemical tests and assays such as immunoassays, comprising a tray of microtitre wells and characterised by comprising in combination a holder bearing a plural number of inserts for the microtitre reaction wells, and a tray having both a main recess for the holder and the inserts borne thereon, and a plurality of reaction wells arranged in a pattern complementary

to the pattern of a plurality of the inserts, so that several of the inserts can be introduced at once into the corresponding reaction wells.

4. Apparatus according to claim 3, characterised by having a holder carrying a row of inserts and a tray with several rows of wells arranged so that the inserts can be introduced successively into each of the rows of wells.

5. Apparatus according to claim 3 or 4, characterised by inserts carried on the holder and wells located in the tray which substantially complement each other in shape so that reaction liquid, when contained in a well in which an insert has been introduced, is constrained to a shell-like distribution between the wall of the well and the insert.

6. Apparatus according to claim 3, 4 or 5, characterised by carrying immunological sensitisation on the surfaces of the inserts, and optional complementary reagents for immunoassay dosed and sterilised in the reaction wells.

7. Apparatus according to claim 6, characterised by a storable form with a sealing cover enclosing both the wells and the inserts and holder located in the main recess.

8. Apparatus according to any of claims 3-7, characterised by complementary locating means on both the holder and the tray, to allow insertion of the inserts into the wells in a desired orientation of match, and to prevent incorrect insertion in the inverse orientation.

9. Apparatus according to claim 1 or 3, substantially as hereinbefore described with reference to Figures 1 to 4 of the accompanying drawings or to Figures 1 to 4 as modified by Figure 5, 7 and 8 or Figures 6, 7 and 8 of the drawings.

10. Use of the apparatus according to any one of preceding claims 1-9 for the performance of immunoassay, including enzyme-linked or fluorescence-linked immunoassay.